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Abstract

Used during an oil spill to minimise the formation of an oil slick, dispersants have negative biological effects on marine model organisms. However, no study has investigated the impacts of dispersants on adult sponge individuals. Here, we examine the effects of water accommodated oil fraction (WAF - oil in seawater), chemically enhanced WAF (CEWAF - oil and dispersant in seawater) and Benzo[A]Pyrene on sponge *Halichondria panicea* at physiological and molecular levels. Sponge clearance rate decreased sharply when exposed to WAF and CEWAF but the oil loading at which the clearance rate was reduced by 50% (ED50) was 39-fold lower in CEWAF than in WAF. Transcriptomic analysis revealed a homogenous molecular response with the greatest number of differentially expressed genes

identified in CEWAF samples (1,461 genes). Specifically, genes involved in stress responses were up-regulated. This study presents evidence that the use of dispersants should be considered carefully in areas where sponges are present.

Introduction

In marine environments, hydrocarbons, released in large quantities during an oil spill, can cause diverse biological impacts including changes to gene expression (Jenny et al., 2016), oxidative stress, decreases in immune function (Hannam et al., 2010) and disruption of development/survival of early life history stages of organisms (Stefansson et al., 2016). Chemical dispersants are often applied to reduce the impact of hydrocarbons to facilitate the dissolution of hydrocarbons and reduce the formation of surface oil slicks (National Research Council, 2005). In the laboratory, water accommodated crude oil fractions (WAFs - seawater with oil) and chemically enhanced WAFs (CEWAFs - seawater with oil and dispersant) are used to produce contaminated seawater to replicate conditions experienced during an oil spill (Aurand and Coelho, 2005). However, many studies using WAFs and CEWAFs have shown that dispersants can themselves be toxic or increase the concentration of hydrocarbon in seawater and amplify hydrocarbon bioavailability for marine organisms (Ramachandran et al., 2004; Rodd et al., 2014). The use of chemical dispersants therefore is controversial (Kleindienst et al., 2015) and further work is needed to understand the impact of dispersant on non-model organisms with important roles in ecosystem function, such as sponges (Luter et al., 2019).

Sponges (phylum Porifera**)** are key filter-feeding organisms in aquatic ecosystems. Marine sponges are significant contributors to the bentho-pelagic coupling thanks to their high filtration rate - they can filter a volume of water equivalent to several time their own

body volume per hour - and actively recycle nutrients including carbon, nitrogen and silica (Leys et al., 2011; Maldonado et al., 2012). Their high filtration rate mean that sponges efficiently bioaccumulate a range of chemicals including polycyclic aromatic hydrocarbons (PAHs) (Batista et al., 2013). However, exposure to PAHs has been shown to inhibit larval settlement in sponges *Crambe crambe* (Cebrian and Uriz, 2007) and *Rhopaloeides odorabile* (Negri et al., 2016) as well as induced DNA damage in *Tethya lyncurium* (Zahn et al., 1983). Exposure to diesel-based WAF also promoted the mitogen-activated protein kinases (MAPK) cell signalling pathway in sponge *Suberites domuncula*, a known stress cell signalling pathway (Châtel et al., 2011). Still, further studies are needed to better understand the full-suite of biological impacts and their relation to hydrocarbon concentration to improve management of accidental oil spills.

The temperate shallow-water demosponge *Halichondria panicea* is an ideal model species to investigate the effects of crude oil based WAF and CEWAF on sponges. *H. panicea* is an encrusting demosponge with a wide geographical distribution able to grow in intertidal environment (Ackers and Moss, 2007). This species is easy to collect and has been widely studied (Barthel, 1988; Riisgård et al., 1993; Witte et al., 1994; Schönberg and Barthel, 1997; Khalaman and Komendantov, 2016; Lüskow et al., 2019 amongst others). *H. panicea* has been reported to filter from to 1.1 to 6.1 mL min^{-1} cm^{-3} (Riisgård et al., 1993, 2016). One ecotoxicological study on *H. panicea* is available, and the study reported that sponge water filtration rate was more than halved after cadmium exposure (Olesen and Weeks, 1994). Building on knowledge of *H. panicea* physiology and ecotoxicology, the purpose of this study is to determine the biological effects of Schiehallion crude oil and dispersed crude oil (prepared with dispersant Slickgone NS) water accommodated fractions on *H. panicea*. Specifically, the effects of WAF and CEWAF on *H. panicea* physiology

(clearance rate) and gene expression (transcriptomics and quantitative PCR) were investigated.

Materials and Methods

Sampling

H. panicea samples (between 1.9 and 3.0 cm^3 in size) were collected at Coldingham bay, located 80 kilometres to the south of Edinburgh (55.89°N, 2.13°W) as it can easily be sampled in the bay at low tide from rocks where it grows in a distinctive yellow encrusting morphotype. Sponges were carefully removed from the rocks with a scalpel and placed into sampling bags filled with freshly collected seawater. Samples were stored in insulated containers and quickly returned to the University of Edinburgh where they were transferred to recirculating seawater holding tanks (volume 50 L) at 10°C. Sponges were fed with *Isochrysis* algae every two days prior to the experimental work. Seawater was also collected at Coldingham bay for the preparation of WAF and CEWAF.

Treatment solution preparation

Schiehallion crude oil (BP) and dispersant Slickgone NS (Dasic International) were used in this study. Schiehallon crude oil is produced at Schiehallion oil field in the Faroe-Shetland channel. The crude oil is characterised by an American Petroleum Institute gravity of 25.2, a sulphur content of 0.46 % and a viscosity of 67 centistokes (cST) at 20° C (BP, 2017). Slickgone NS is one of the dispersants approved for use by the United-Kingdom Marine Management Organisation and is listed for potential use in the Faroe-Shetland channel in the case of a spill (BP, 2014; Marine Management Organisation, 2018). To prepare WAF and CEWAF solutions, we followed the standard protocol developed by the Chemical Response to Oil Spills: Ecological Research Forum (Aurand and Coelho, 2005). Weighed amounts of

Schiehallion crude oil was added to 1 L seawater solutions and mixed for 18 h at a speed of 180 rpm for the WAF solutions (to avoid the formation of a vortex) and at a speed of 300 rpm for the CEWAF solutions (to allow the formation of a small vortex) as suggested by Aurand and Coelho (2005) . For CEWAF solutions, dispersant Slickgone NS was applied at a volume ratio of 1:10 as advised by the manufacturers. At the end of the mixing time, solutions were left to settle for 3 h and water fractions were carefully removed and placed into clean 1 L Duran bottles. WAF and CEWAF solutions are characterised by complex mixtures of diverse hydrocarbons which does not easily allow for cross study comparisons. Therefore, a single hydrocarbon treatment was added to the experiment and Benzo[A]Pyrene was selected as it is known for its mutagenic and carcinogenic effects (Liu et al., 2010). To produce a solution of BaP in seawater at a final concentration of 10 µg/L, BaP was first dissolved in dimethyl sulfoxide (DMSO) before being added to the seawater (final dilution 0.01% v/v). To exclude any effect of the DMSO in the BaP treatment, a DMSO treatment (0.01% v/v) was also added. For the BaP solution, a stock solution of BaP in DMSO at a concentration of 0.1 g/L was first prepared. 100 µL of stock solution was then added to 999.9 mL of artificial seawater prior to the use in the experiment. The same volume of DMSO was also added to artificial seawater in the DMSO solution.

Experimental design

To investigate the impacts of WAF and CEWAF on *H. panicea*, two experiments were conducted: a single concentration experiment and a dose-response experiment. In both cases, a flow-through experimental apparatus was used. The experimental apparatus was constituted of 12 individual glass incubation chambers of 750 mL of volume, sealed with a polytetrafluoroethylene lid. Each chamber was equipped with a magnetic stirrer at the top of the chamber. An inflow and outflow in the lid allowed seawater to flow through the chamber

at a rate of 750 mL/day, thanks to multichannel peristaltic pumps (figure S1). PTFE tubing connected the chambers to the peristaltic pumps to limit to a maximum the use of plastic (which would interact with hydrocarbons). One individual sponge sample was placed in each incubation chamber at the beginning of the experiments. All experiments were conducted in the dark to avoid photo oxidation and in a temperature controlled room (10° C).

In the single concentration experiment, sponge samples (three sponges per treatment) were exposed, for 48 h, to control seawater (seawater without any contaminants), to WAF (1.0 g/L of oil loading) and to CEWAF (1.0 g/L of oil loading with dispersant). Sponge samples (three sponges per treatment) were also exposed to BAP in DMSO (10 µg/L; 0.01% v/v) and DMSO (0.01% v/v) to be used as positive and negative control samples. First, samples were placed in their incubation chambers and left to acclimatise in control seawater for 48 h. Sponges were then exposed for 48 h to the relevant treatment. Control seawater was finally pumped back into the chambers for 48 h to test for any recovery potential.

In the dose-response experiment, new sponge samples were exposed for 48h to increasing nominal concentrations of crude oil (WAF dose-response) or crude oil and dispersant (CEWAF dose-response). In both cases, the following nominal oil loading were tested: 0.01 g/L, 0.03 g/L, 0.05 g/L, 0.1 g/L, 0.5 g/L, 1.0 g/L, 3.5 g/L, 5.0 g/L, 7.5 g/L and 10.0 g/L. Between 1 and 6 replicates were included for each nominal oil loadings as equilibrated experimental designs are not needed for the non-linear regression modelling approach used in this study (see below, statistical analysis; Ritz, 2010). Six replicate controls (sponges kept in seawater) were also included in each experiment. Sponges were placed in incubation chambers and left to acclimatise for 48 h before exposure, as in the single

concentration experiment. The sponges were then exposed to treatments for 48 h after which the dose-response experiment was terminated.

Analytical chemistry

GC-MS analysis of a subset of water samples collected from the incubation chambers at the end of each experiment was conducted to determine what PAH concentrations the sponge was exposed to. Collection of 100 mL water samples occurred at the end of each exposure directly from the individual incubation chambers. Molecular grade dichloromethane (10 mL) was used as the organic phase for liquid-liquid extraction of the hydrocarbons from water samples. A rotary evaporator was then used to remove the organic solvent. After sample preparation, the extracts were diluted in 0.5 mL of dichloromethane. The chromatographic analysis of 16 PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[A]Anthracene, Chrysene, Benzo[B]Fluoranthene, Benzo[K]Fluoranthene, Benzo[A]Pyrene, Indeno[1,2,3,C,D]Pyrene, Dibenz[A,H]Anthracene and Benzo [G,H,I]Perylene) was carried out on a GC-MS equipment (Shimadzu, model QP-5050 A) based on the USEPA 8270 method using a capillary column DB-5MS (J&W Scientific): 30 m x 0.25 mm ID x 0.25 µm film thickness. A 1 µL aliquot of the final extract was injected in the split mode. Data acquisition was performed in the SIM mode. Each PAH was individually quantified using a (1-1000 µg/L) calibration curve obtained after the appropriate dilution of an analytical standard solution (48743 Supelco, EPA 610 PAH Mixture). The limit of quantification in the hydrocarbon extracts (LOQ) varied between 1 and 10 µg/L and the correlation coefficients of each individual PAH analytical curve were above 0.99.

Clearance rate determination

Clearance rate is an estimation of the amount of water filtered by the sponge per unit of time and can be used as a proxy to assess the sponge pumping activity. In this study, measurements of clearance rate occurred before, during and after exposure in the single-concentration experiment and at the end of exposure in the dose-response experiment. As described in Riisgård et al. (2016), clearance rate is measured by following the exponential decrease of algea cells over time. Therefore, to assess clearance rate, a diluted solution of *Isochrysis* Instant Algae® (Reed Mariculture) was added to each chamber (for a final algae concentration in the incubation chambers of 4,000 – 5,000 cells/mL (Kumala et al., 2017)) and the sponges were left to filter for 2 h. Stirring in the chambers was kept active during the measurements but the peristaltic pumps were stopped so that no fresh input of seawater was added. Water samples were collected every 20 min and algae concentrations in each sample were determined spectrophotometrically through total absorbance measurements at 750 nm using a previously prepared calibration curve (Griffiths et al., 2011). To standardise the clearance rate by the sponge volume, the thickness and surface area of each sample was determined at the end of the experiments with the freely available software Fiji (Schindelin et al., 2012). The clearance rate (CR in cm^3 min^{-1} cm^{-3}, i.e. cm^3 of water cleared per min per cm^3 of sponge tissue) was then calculated using equation (1):

$$(1)\ CR = \frac{\frac{V_{SW}}{t} \ln\frac{C_0}{C_t}}{V_{sp}}$$

Where C_0 and C_t are the initial and final concentration of algae, V_{sw} and V_{sp} are the volumes of seawater and sponge tissue respectively, and t is time.

To determine if the treatment influenced clearance rates during and after exposure in our single concentration experiment, a repeated-measure Analysis of Variance (ANOVA) was carried out with the package lme4 (version 1.1-21; Bates et al., 2015). To determine the

effect of increasing oil loading in WAF and CEWAF solutions on clearance rate (dose-response experiment), a dose-response analysis was performed using the package drc (version 3.0-1; Ritz et al., 2015). A dose-response model was fitted to the data using a Weibull 1 three parameter function defined by equation (2):

$$(2)\ f(x,b,d,e) = d\ exp\left(-exp\left(b\left(log(x) - log(x)\right)\right)\right)$$

Where b is the slope of the dose-response curve, d the upper asymptote, and e the efficient dose ED50 (Ritz et al., 2015). To assess if the dose-response model constructed was significant and fitted the data well a no effect and lack-of-fit tests were carried out (Ritz et al., 2015). A parameter comparison test was then performed to detect any statistically significant differences between the slope, upper asymptote and ED50 values between the WAF and CEWAF treatments (Ritz et al., 2015). Although the ED50 (defined as the nominal oil loading necessary to drop the sponge clearance rate by half) is a useful parameter to compare the effects of WAF and CEWAF on the *H. panicea* clearance rate in this study, it doesn't allow for comparisons with other studies and other filter-feeding organisms. Therefore, the ED50 was also converted to the EC50 (defined here as the total concentration of the 16 PAH (ΣPAH_{16}) necessary to drop the sponge clearance rate by half) by using the GC-MS data produced in this study.

RNA extraction

Total RNA was extracted from tissue samples collected at the end of the exposure in all experiments. Total RNA was extracted from sponge tissue samples using Qiagen Total RNA Blood and Tissue Extraction kits following the manufacturer's protocols. DNA was removed from the RNA extractions with Qiagen DNase sets. At the end of the protocol, RNA was eluded into 30 µL of DNA/RNA free sterile water. RNA quality and quantity were then

assessed by spectrophotometer using a NanoDrop. Only RNA samples with 260/230 and 260/280 ratios of 1.8-2.2 were retained for further analysis.

Transcriptomics

RNA extraction from all twelve samples of the single exposure experiment were sent to Edinburgh Genomics for Illumina TruSeq stranded mRNA-seq library preparation and Illumina HiSeq 4000 RNA-sequencing. Paired-end reads were run to 75 bp to yield at least 290 M + 290 M reads. Upon reception of the sequencing data, Illumina adaptors were trimmed, and the quality of the reads was checked using Fastqc (version 0.11.5; Andrews, 2015). Reads under a quality Phred score of 30 were removed. Reads from all twelve sequenced samples were pooled and uploaded to the main Galaxy web-platform (Afgan et al., 2018) to construct a Trinity *de novo* assembly (version 2.2.0; Grabherr et al., 2011). Contigs less than 300 bp were removed from the *de novo* assembly. To assess assembly completeness, BUSCO was run on Galaxy against the eukaryote_odb9 and metazoan_odb9 datasets (Kenny et al., 2018; Seppey et al., 2019). Transcripts were then quantified by RSEM (Li and Dewey, 2011) and a non-metric multidimensional scaling (nMDS) ordination analysis of normalised expression levels from each sample was performed. A differential expression analysis was conducted with the R package edgeR (version 3.24.3; Robinson et al., 2010) and comparisons were considered significant at an FDR adjusted value of 0.01. A BLASTx search was thereafter performed for all differentially expressed genes against the NCBI *nr* protein database, using a e-value cut-off of 1e-5. Gene Ontology terms (GO) was retrieved from the UniProt database for differentially expressed genes with BLAST hits. A GO enrichment analysis using a Fisher exact test and an 'elim' algorithm was finally performed on the down- and up- regulated genes separately using the R package TopGO (version 2.34.00; Alexa and Rahnenfuhrer, 2016). Expression levels of genes of interests

were plotted using the function heatmap.2 from the R package gplots (version 3.0.1.1; Warnes et al., 2019).

Gene expression through quantitative PCR

To support the results of the transcriptomic analysis done on the single-exposure experiment and develop gene biomarkers of sponge stress response, gene expression analysis on dose-response samples via quantitative PCR (qPCR) was undertaken. Target genes coding for cyclophilin, cytochrome b5 and heat shock protein (hsp) 70 were selected from the transcriptomic data to develop qPCR primers (table S1) because of their known role in cellular detoxification pathways (Schröder et al., 1999; Boutet et al., 2004; Webster et al., 2013). A reference gene coding for a tyrosine kinase which was not found to be differentially expressed in the transcriptomics study was also chosen (table S1). For RNA samples originating from the dose-response experiments, RT-PCR was performed using nanoScript™ 2 Reverse Transcription kits following manufacturer's instructions. RNA samples were diluted to a concentration of 100 ng/µL prior to the RT-PCR. The annealing step of the RT-PCR was conducted at 65° C for 5 min. After that, the extension step was carried out in 30 cycles of 42° C for 20 min followed by 75° C for 10 min. cDNA samples were stored at -20° C until qPCR was performed. qPCRs of cDNA samples from the dose-response experiments were undertaken using Primer Design PrecisionPLUS mastermix kits following manufacturer's protocol. Relative fold change in expression of each target gene was then determined using the $\Delta\Delta C_t$ method (Henry et al., 2009).

Results

Analytical chemistry

GC-MS analysis of water samples was performed to determine the concentration of 16 PAHs. Overall, hydrocarbon concentrations increased in our samples with addition of dispersant in the single concentration experiment (Table S2) and with increasing oil loadings in the dose-response experiment (Tables S3 and S4). In the single concentration experiment, individual PAH concentration ranged from < LOQ to 0.05 µg/L in the WAF treatment and from < LOQ to 0.07 µg/L in the CEWAF treatment (Table S2). ΣPAH_{16} reached $1.19x10^{-1} \pm 3.74x10^{-2}$ µg/L in the WAF treatment and $2.19x10^{-1} \pm 3.41x10^{-2}$ µg/L in the CEWAF treatment (Table 1). In the dose-response experiments, a broad test range of hydrocarbon concentrations was achieved with increasing oil loadings and addition of dispersant in the CEWAF treatment as individual hydrocarbon concentration measured varied from < LOQ to $1.70x10^{3}$ µg/L (10.0 g/L of oil with dispersant; Tables S3 and S4). Only samples with very high oil loading and dispersant reached the upper concentration ranges displayed in the data. Complex aromatics such as benzo[B]fluoranthene and benzo[K]fluoranthene were found in CEWAF samples at high oil loading alone (7.5 g/L and 10.0 g/L of oil with dispersant; Table S4). Dibenz[A,H]anthracene and benzo[G,H,I]perylene, the most complex compounds considered in our assay, were not detected in any of our samples (Tables S2 to S4). Individual PAH concentration as well as ΣPAH_{16} were generally higher in the CEWAF treatment than in the WAF treatment across both experiments. For example, at an oil loading of 0.5 g/L in the dose-response experiment, ΣPAH_{16} reached $1.54 \pm 1.25x10^{-1}$ µg/L in the WAF treatment and $7.71x10^{1} \pm 1.44x10^{1}$ µg/L in the CEWAF treatment (Table 1).

Gross observations

All *H. panicea* samples kept in control conditions survived the experiments, appeared healthy and still displayed their sharp yellow colours at the end of the experiments. Furthermore, all samples survived the 48 h exposure to DMSO, BaP and WAF during the single concentration experiment and the 48 h exposure to WAF in the dose response experiments, except for the highest oil loading tested (10.0 g/L; ΣPAH_{16} not available). When considering the CEWAF treatment, all sponges survived the exposure in the single concentration experiment (1.0 g/L of oil with dispersant; $\Sigma PAH_{16}=2.19x10^{-1} \pm 3.41x10^{-2}$ µg/L). However, some samples exposed to the highest oil loading in the CEWAF dose response experiment rapidly turned dark in colour and died within the first 24 h of the exposure. Specifically, sponges exposed to 3.5 g/L (ΣPAH_{16} not available), 7.5 g/L (one of the two replicates survived; $\Sigma PAH_{16}=1.98 \pm 1.91x10^{-1}$ µg/L) and 10.0 g/L of oil with dispersant ($\Sigma PAH_{16}=1.38x10^{4}$ µg/L) did not survive the exposure.

Physiology

Clearance rates varied between individuals throughout the single concentration experiment, but a clear pattern could be detected (figure 1). All values measured in control and DMSO samples across the experiment as well as in samples before exposure in the hydrocarbon treatments ranged between 0.5 and 4.9 $cm^3\ cm^{-3}\ min^{-1}$. A sharp decrease in clearance rate was measured in all samples exposed to WAF, CEWAF and BaP during the exposure and the clearance rate remained low 48 h after the end of the exposure (figure 1). In the hydrocarbon treatment conditions (WAF, CEWAF and BaP) during and after exposure, clearance rate decreased to a minimum of 0.1 $cm^3\ cm^{-3}\ min^{-1}$. Time (*p*-value=1.61e-08) and Treatment*Time (*p*-value=3.01e-05) appeared strongly statistically significant in the repeated-measures ANOVA (table S5).

Clearance rate data gathered in the dose-response exposure experiment was in accordance with the data collected during the single concentration experiment. Clearance rate rapidly decreased with increasing oil loading in both the WAF and CEWAF treatment of the dose-response experiment (figure 2). Clearance rate in control conditions reached a maximum of 4.9 cm^3 cm^{-3} min^{-1} across WAF and CEWAF experiments (figure 2). Clearance rate in the WAF exposure dropped to a minimum of 0.3 cm^3 cm^{-3} min^{-1} (10 g/L of oil loading) while falling to a minimum of 0.01 cm^3 cm^{-3} min^{-1} in the CEWAF experiment (5 g/L oil loading). Overall, at a same oil loading, clearance rates in samples exposed to CEWAF were lower than those measured in WAF. All coefficients of the Weibull 1 dose response model were statistically significant (table S6). The model fitted the data well (lack of fit test *p*-value=0.84) and a dose effect on clearance rate was statistically significant (*p*-value=6.4e-07) (table S6). The WAF ED50 reached 1.56 g/L of oil loading which is equivalent to ~1.6 µg/L ΣPAH_{16} based on our GC-MS data. CEWAF ED50 reached 0.04 g/L of oil loading which is equivalent to ~1.0 µg/L ΣPAH_{16} based on our GC-MS data. This means that the oil loading in the WAF treatment needed to lower by half the clearance rate of *H. panicea* is 39-fold higher that in the CEWAF treatment. The parameter comparison test determined that the CEWAF ED50 was statistically different from the WAF ED50, while the slopes for each treatment did not differ significantly (table S7).

Transcriptomics

Transcriptomic analysis of samples collected after exposure in the single concentration experiment was performed. RNA sequencing yielded an average of 47 million reads of 75 bp per treatment after trimming (table S8). The Trinity *de novo* assembly constructed in this study was composed of 235,561 contigs with a N50 of 984 bp and a GC

ratio of 45.54. The assembly average contig size and total assembly length reached 783 and 227,885,271 bp respectively (table S9). The BUSCO analysis revealed that the *de novo* assembly was complete against both the eukaryotic and metazoan datasets. All 303 genes from the eukaryote_odb9 dataset were identified in the assembly (53 complete and single-copy BUSCOs, 250 complete and duplicated BUSCOs) leading to a completeness of 100%. 951 out of 978 genes from the metazoan_odb9 dataset were found in the assembly (263 complete and single-copy BUSCOs, 688 complete and duplicated BUSCOs, 17 fragmented BUSCOs and 10 missing BUSCOs) leading to a completeness of 97.2%. Overall, significant differences in gene expression patterns between control-DMSO samples and WAF-CEWAF-BaP samples were detected despite noticeable inter-individual variations (Figure 3). In total, 1,917 genes were found to be statistically differentially expressed (FDR adjusted value cut-off of 0.01) across all treatments compared to control samples. The largest number of differentially expressed genes (1,461) was detected in the CEWAF treatment while the smallest number of differentially expressed genes (237) was found in the DMSO treatment compared to controls (figure 4). 472 genes were only detected as differentially expressed in the CEWAF treatment (figure 4). The distribution of log fold changes (logFC) values for all differentially expressed genes from the CEWAF samples were further investigated. Figure 5 shows the ordered logFC of the 1,461 CEWAF differentially expressed genes compared to the logFC of the same genes in the other treatments. Most differentially expressed genes considered behaved similarly across all hydrocarbon treatments (WAF, CEWAF and BaP) (figure 5AB). This is in sharp contradiction with what can be observed in the DMSO treatment. Most differentially expressed genes identified in the CEWAF treatment appear not to be differentially expressed in the DMSO treatment (figure 5C).

Specific genes of interest were identified amongst the pool of differentially expressed genes. BLAST of the 1,917 differentially expressed genes in our experiment returned hits for 32% of the contigs. Of the identified gene pool, genes involved in the regulation of oxidative stress, in the control of cell cycling, in stress response and MAPK pathway were identified (table 2). A dendogram and a heatmap were constructed showing the expression levels of the 30 genes highlighted in table 1 across experimental samples (figure 6). Although strong inter-individual variations can be observed in the heatmap, the dendrogram classification allocated samples into three clusters, reflecting the results of the nMDS ordination analysis (figure 3): (1) control and DMSO samples, (2) WAF samples, CEWAF sample 2 and 3 as well BaP1 and 3, (3) CEWAF 1 and BaP3 (figure 6). Overall, members of the TNF family, involved in the regulation of the immune system, were often found to be highly expressed in the control samples. On the contrary, in WAF, CEWAF and BaP samples, universal stress proteins, heat shock proteins and member of the cytochrome b and c families were expressed at high levels (figure 6). Gene ontology (GO) analysis was performed separately on the down- and up-regulated gene pools identified from the hydrocarbon treated samples compared to the controls and identified from Porifera, Metazoa and Eukaryota BLAST hits. The GO analysis on the up-regulated gene pool retained five statistically significant GO terms: defence response (GO:0006952), biological and cell adhesion (GO: 0022610 and GO:0007155), one-carbon metabolic process (GO:0006730) and cellular macromolecule catabolic process (GO:0044265; figure S2). The GO analysis on the down-regulated gene pool retained DNA metabolic process (GO:0006259) as the only statistically significant GO term.

Gene expression

No significant differences in the mean C_T values for the gene TK were detected between treatment or time points. Therefore, TK could be used as a reference gene in the

calculation of $\Delta\Delta C_t$ values for the genes of interest. As expected from the transcriptomics analysis, all three target genes (cyclophilin, cytochrome b5 and hsp70) were up-regulated in WAF and CEWAF samples from the lowest to the highest oil loading so from ΣPAH_{16}=8.04x10^{-2} ± 6.69x10^{-3} µg/L (WAF 0.01 g/L of oil) to ΣPAH_{16}=1.98 ± 1.91x10^{-1} µg/L (CEWF 7.5 g/L of oil with dispersant). High variation in logFC between samples was, however, observed. For cyclohilin, logFC in exposed samples varied between 1.27 and 24.7. For cytochrome b5, logFC in exposed samples varied between 1.29 and 16.6. For hsp70, logFC in exposed samples varied between 1.02 and 13.0. No dose-response relationship between the logFC and the oil loading was found in either the WAF or CEWAF treatments.

Discussion

Treatment conditions tested in this study

In the single exposure experiment, BaP in DMSO and DMSO alone treatments were tested in addition to the WAF and CEWAF treatments. BaP is a PAH known to have significant cytotoxic properties in a range of organisms (Regoli and Giuliani, 2014) and is considered in this study as a positive control treatment. Exposure to BaP at the concentration used here (10 µg/L) had previously been studied in the sponge *Tethya lyncurium* and led to DNA damage (Zahn et al., 1981, 1983). Similarly, the DMSO treatment included in the single concentration experiment was considered as a negative control in our study. The role of the BaP treatment as a positive control and of the DMSO treatment as a negative control were both confirmed by the transcriptomics data. Contrary to samples in the DMSO treatment, sponges exposed to BaP shared a similar gene expression profile with samples exposed to WAF and CEWAF. Some differentially expressed genes were still detected when comparing DMSO and control treatments. This is most likely due to the high inter-individual variability found in our study in both the physiological and molecular datasets. However, the nMDS and clustering analysis of transcriptomics data both revealed that DMSO and controls

could be grouped together compared to the hydrocarbon treatments. Therefore, the use of BaP and DMSO treatments in this study can enable further laboratory studies to be conducted on the uptake and accumulation of hydrocarbons in sponges, which is at the moment only known through the gathering of environmental samples (Batista et al., 2013).

Differences between CEWAF and WAF hydrocarbon content due to the addition of dispersant were detected in our study. GC-MS analysis of water samples from the experiments detected higher concentrations of individual hydrocarbons in the CEWAF treatment than in the WAF treatment at the same oil loading. This confirms the effectiveness of dispersants to increase the amount of hydrocarbons entrained into the seawater (both dissolved and in droplets) (Fingas, 2001). Although the impact of dispersant alone could have been tested in this study, it seemed more appropriate to determine the impact of dispersed oil on *H. panicea*. The toxicity of dispersants to various organisms has been widely discussed in the scientific literature over the years (Singer et al., 1998; Shafir et al., 2007; Zhang et al., 2013; DeLeo et al., 2016) but exposure to dispersant alone is not very likely. Exposure to a mixture of dispersant and oil will reflect more accurately an oil spill scenario where dispersants are applied to an oil slick.

Individual hydrocarbon concentrations measured in the single concentration experiment and at low oil loadings in the dose response experiment were overall lower than those described in the CROSERF protocol followed to produce the water accommodated solutions (Aurand and Coelho, 2005). This difference was most likely due to (1) the larger incubation chamber used in our study to allow experimentation on sponges, and (2) biodegradation of hydrocarbon over time. All our experiments started with a 48 h acclimatisation phase during which sponges were kept in the incubation chambers in

seawater. The relevant treated seawater was then slowly pumped into the chamber leading to the dilution of WAF and CEWAF solutions. Bacterial degradation of hydrocarbons over time might also have contributed to the lower hydrocarbon concentration observed (Head et al., 2006). Despite being lower than the values reported in the CROSERF protocol, concentration measured in our samples in the single exposure experiment (ΣPAH_{16} 0.08 to 0.25 μg/L across the WAF and CEWAF treatment) are below the concentration measured in surface seawater during the Deepwater Horizon oil spill (ΣPAH_{16} 0.88 to 6.28 μg/L) (Diercks et al., 2010).

In the dose response experiment, a large range of WAF and CEWAF solutions with increasing oil loadings up to 10.0 g/L were tested. The highest range of oil loadings were not intended to represent realistic oil spill scenarios (and this was confirmed by the high levels of individual hydrocarbon concentrations) but were intended to determine the maximum oil loading and ΣPAH_{16} concentrations that *H. panicea* could survive for a short exposure period of 48 h. In this study, lethal effects were detected at oil loadings as low as 3.5 g/L (ΣPAH_{16} $<5.57 \times 10^2$ μg/L) in the CEWAF experiment but not until the highest oil loading of 10.0 g/L (ΣPAH_{16} $>26.76 \pm 6.78$) in the WAF experiment. These findings suggest that *H. panicea* is resilient to crude oil contamination alone during short exposure periods but sensitive to the use of dispersant.

Physiology

Clearance rate in control and pre-exposure conditions across both the single exposure and the dose-response experiment displayed large inter-individual variability. However, these values of clearance rate obtained in control conditions fit within the literature available for *H. panicea* (Riisgård et al., 1993) and for other encrusting sponges (De Goeij et al., 2008).

Although care was taken in selecting samples of similar size and normalising the clearance rate by the sponge volume, clearance rate of an individual sponge will most probably vary depending on its number of oscula (Kumala et al., 2017). Further studies selecting samples with the same number of oscula or on single osculum explants could help refine the results found in this study.

Despite this inter-individual variability, sponges exposed to hydrocarbons treatments (WAF, CEWAF and BaP) displayed significantly diminished clearance rate in the single exposure experiment. A decrease in filtration rate was also observed in *H. panicea* exposed to cadmium (Olesen and Weeks, 1994), suggesting that this could be a typical physiological response to pollutants in *H. panicea.* Concentration of cadmium above 100 µg/L lead to longer term filtration loss with filtration rate remaining low for several hours after exposure (data only available for 4 h in the study; Olesen and Weeks, 1994). This is also in accordance with observations made in the single exposure experiment where sponges exposed to hydrocarbon treatments displayed lowered clearance rates even 48 h after the end of the exposure time. It is likely that stopping its filtration activity for extensive periods of time will strongly impact survival of *H. panicea*, by lowering the energy input the sponge receives (Grant et al., 2018). The capacity of sponges to survive longer exposure periods should therefore be investigated.

In the dose-response experiments, clearance rate decreased with increasing oil loading, with or without dispersant. However, the clearance rate ED50 in the CEWAF treatment was 39-fold lower than in the WAF samples. Furthermore, the clearance rate EC50 was found to be ~1.6 µg/L ΣPAH_{16} in the WAF treatment while only reached ~1.0 µg/L ΣPAH_{16} in the CEWAF treatment. Although it is important to keep in mind that the low

values observed for both EC50 could be due to dilution and biodegradation of oil in the seawater, the difference in ED/EC50 between WAF and CEWAF is most likely due to the addition of dispersant in the CEWAF treatment. As shown by the analytical chemistry data, CEWAF samples were characterised by higher concentrations of PAH. This could explain the lower ED/EC50 and ED/EC50 for clearance rate measured in sponges exposed to CEWAF compared to WAF and demonstrate the sublethal negative impact of dispersant on *H. panicea*.

Gene expression

Differential expression analysis of transcriptomic data revealed that sponges exposed to the CEWAF treatment displayed the largest number of differentially expressed genes relative to control. The use of dispersants increased both the concentrations of hydrocarbons in seawater which led to a heightened molecular response in *H. panicea*. Identification by BLAST of many of these differentially expressed genes was, however, impaired by the low number of Porifera genes available in the NCBI database (Riesgo et al., 2012).

Our study shows that when exposed to hydrocarbons, expression of genes involved metabolic pathways are greatly altered. GO enrichment analysis revealed that genes involved in DNA metabolic processes were found down-regulated in samples exposed to hydrocarbons compared to controls while genes involved in the defence response, cell adhesion, one-carbon metabolic pathways and macromolecule catabolic processes were found to be up-regulated. This is probably in response to the diminished clearance rate observed in the physiological dataset leading to a decrease in energy intake. Energy intensive house-keeping functions such as DNA replication are down-regulated while genes involved in general detoxification pathways and stress response are up-regulated. This response is consistent with other studies

on sponges (Guzman and Conaco, 2016) and other aquatic organisms (Leveelahti et al., 2011; Jenny et al., 2016) exposed to chemical and thermal stressors or nutrient deprivation (Fan et al., 2020).

Cellular response to hydrocarbon exposure is often mediated through the aryl hydrocarbon receptor (AHR) pathway (Puga et al., 2002; Jenny et al., 2016). Target of the AHR pathway include both Phase I and Phase II detoxification genes and may also cause changes to the cell cycle (Puga et al., 2002). Other key cell signalling pathways are the MAPK and JNK cell signalling pathways, which were shown to be activated in the sponge *S. domuncula* exposed to diesel oil WAF (Châtel et al., 2011) and in sponge *R. odorabile* exposed to thermal stress (Webster et al., 2013). Although no clear signalling pathway could be identified in our analysis, genes involved in AHR, MAPK and JNK cell signalling pathways were identified. Specifically, heat shock proteins, cyclophilins, cytochromes b and c, all involved in xenobiotic detoxification pathways, were found to be up-regulated in the hydrocarbon treated samples. Heat shock genes code for highly conserved proteins, which act as molecular chaperones (Liu et al., 2015a). Specifically, Hsp70 and Hsp90 play a key role in the metabolic detoxification of toxic stressors including PAHs, pesticides or heavy metals (Regoli and Giuliani, 2014) and were also found up-regulated in sponge *R. odorabile* larvae exposed to WAF and CEWAF (Luter et al., 2019). Hsp70 help to clear aggregated proteins and suppress the ability of cells to undergo apoptosis (Liu et al., 2015a). As such, Hsp70 is part of the phase II of the detoxification process (Regoli and Giuliani, 2014). Hsp90 forms a dimer with the AHR, which dissociates when the xenobiotic enters the cell (Regoli and Giuliani, 2014; Liu et al., 2015a). After dissociation, the AHR primes the translocation of genes involved in the part I of the detoxification process (Liu et al., 2015a) such as Cytochrome b5. Cytochrome b5 is part of the cytochrome P450-dependent monooxygenase

system, a key part of the phase I of the detoxification process (Porter, 2002). Cyclophilins are also a group of structurally conserved cytosolic proteins characterised by a peptidyl-prolyl isomerase activity (Wang and Heitman, 2005). Specifically, Cyclophilin A was found upregulated in the plant model *Arabidopsis thaliana* exposed to the PAH phenanthrene (Liu et al., 2015b) and regulates the JNK and p38 MAPK signalling pathways (Kim et al., 2015).

Detoxification genes up-regulated in the single concentration experiment were also found up-regulated through qPCR in the samples from the dose-response experiment. The aim of our qPCR analysis was to develop primers for genes that could be used as reliable biomarkers of stress in sponge *H. panicea*. However, no dose response between the logFC and the oil loading was found for any of the genes tested (Hsp70, cyclophilin and cytochrome b5) and a high level of inter-individual variability was also observed, consistent with the transcriptomic expression values seen in the heatmap. The lack of dose response relationship between gene response and oil loading could be due to the fact that the samples were taken at the end of the 48 h exposure time and might have been detected in samples taken a few hours after the beginning of the exposure. Characterising the time response of Hsp70, cyclophilin and cytochrome b5 is needed to determine if these genes can be used in future studies as biomarkers of stress in *H. panicea*.

Conclusion

In this study, we show that *H. panicea* exposed to CEWAF displayed the strongest decrease in clearance rate as well as the largest numbers of differentially expressed genes compared to any other hydrocarbon treatment tested and led to mortality of some sponge samples. These effects are most likely due to the increase in complex PAH concentrations

following the application of dispersant. The use of dispersants in area rich in sponges should therefore be avoided in the future.

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Data Availability

The GC/MS data as well as the quantitative PCR data referred to in this study are available on PANGEA at https://doi.pangaea.de/10.1594/PANGAEA.911490 and https://doi.pangaea.de/10.1594/PANGAEA.911489.
The transcriptomic sequences used in this study are available on ENA under accession codes ERX4118252 to ERX4118266.

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Zhang, Y., Chen, D., Ennis, A.C., Polli, J.R., Xiao, P., Zhang, B., Stellwag, E.J., Overton, A., Pan, X., 2013. Chemical dispersant potentiates crude oil impacts on growth, reproduction, and gene expression in *Caenorhabditis elegans*. *Arch. Toxicol*. 87, 371–382. https://doi.org/10.1007/s00204-012-0936-x

Figures and Tables:

Figure 1: Clearance rate of sponge samples before, during and after exposure for each treatment considered in the single exposure experiment. In the first graph, open symbols represent DMSO samples while closed symbols represent control samples.

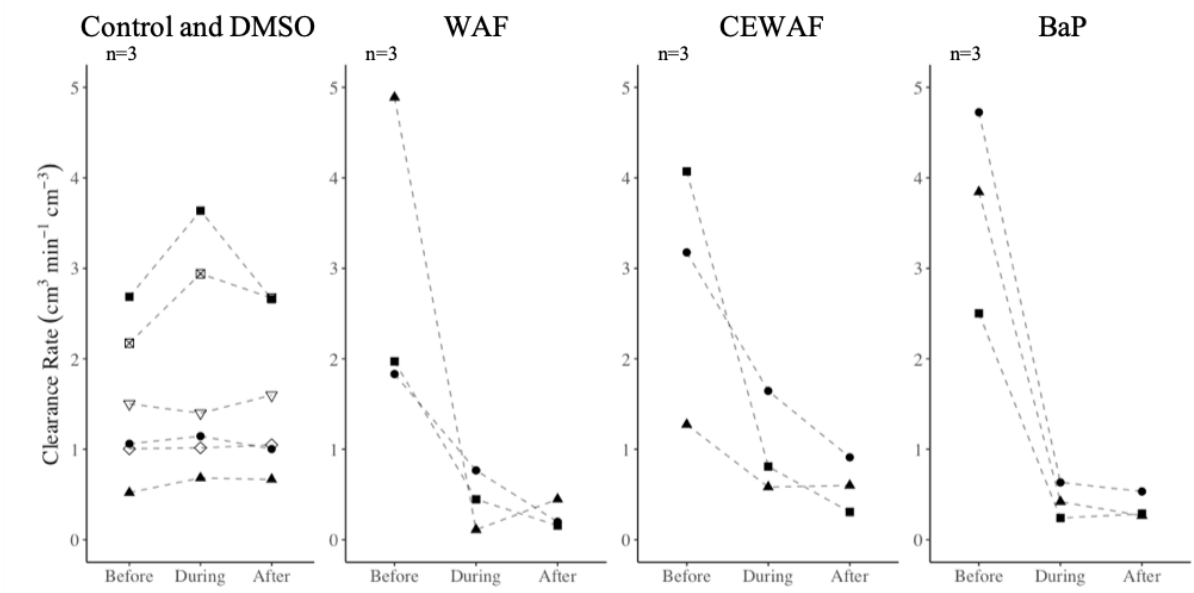

Figure 2: Dose-response physiology measurements. WAF measurements are in black dots or CEWAF measurements are in red squares. The clearance rate dose-response model (Weibull 1 three parameter model) has been plotted in black for the WAF treatment and in red for the CEWAF treatment. Ribbons around each curve represent the 95% confidence intervals.

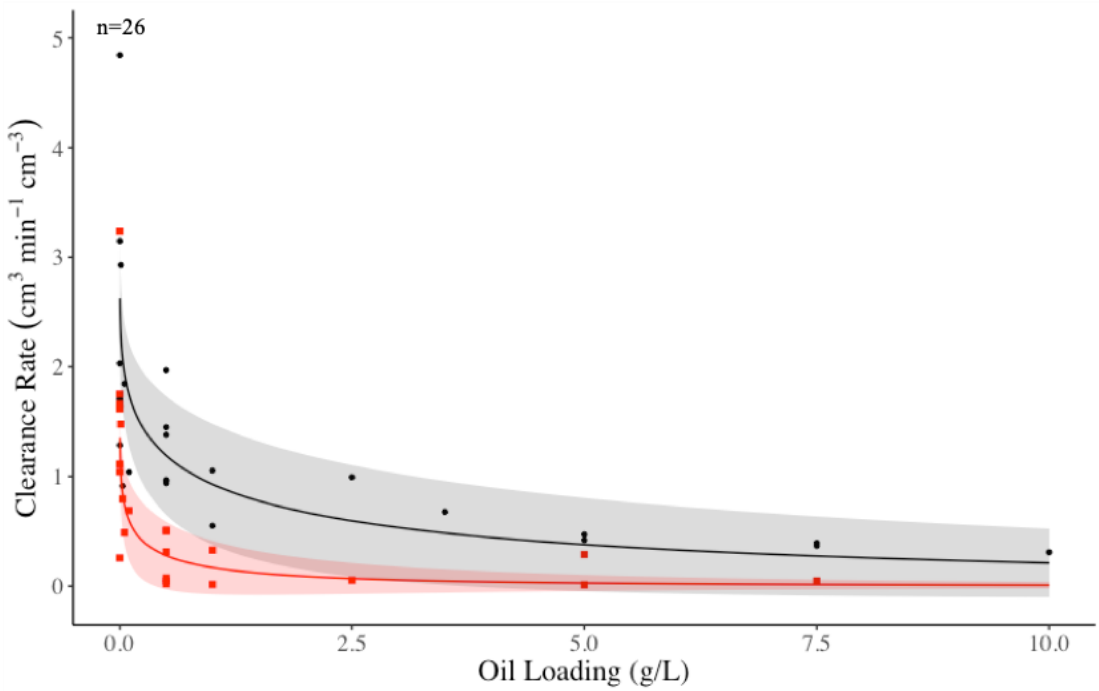

Figure 3: Non-metric multidimensional scaling ordination plot of normalised gene expression data across samples.

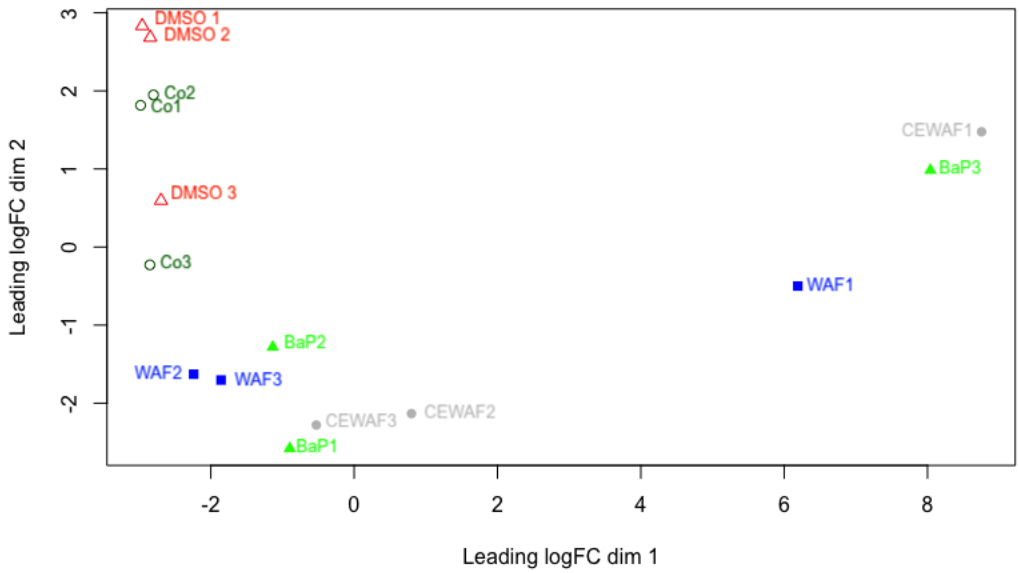

Figure 4: Venn Diagram of the number of differentially expressed genes in each treatment compared to control.

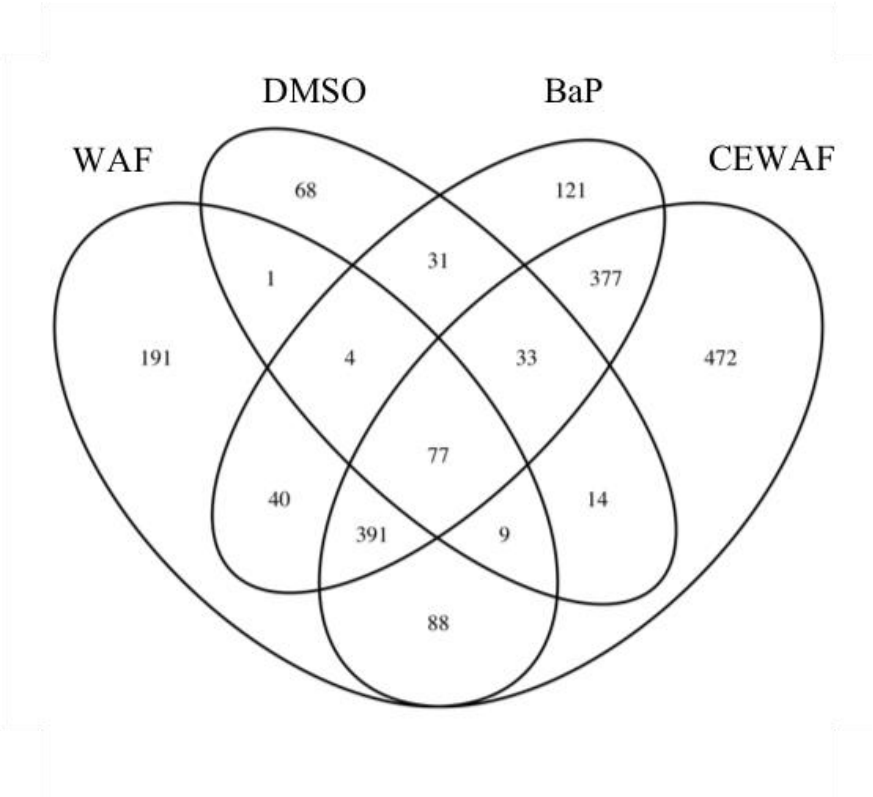

Figure 5: Plot of the ordered differentially expressed genes relative to control in the CEWAF treatment (black dots) compared to (1) WAF, (B) BaP or (C) DMSO (red crosses).

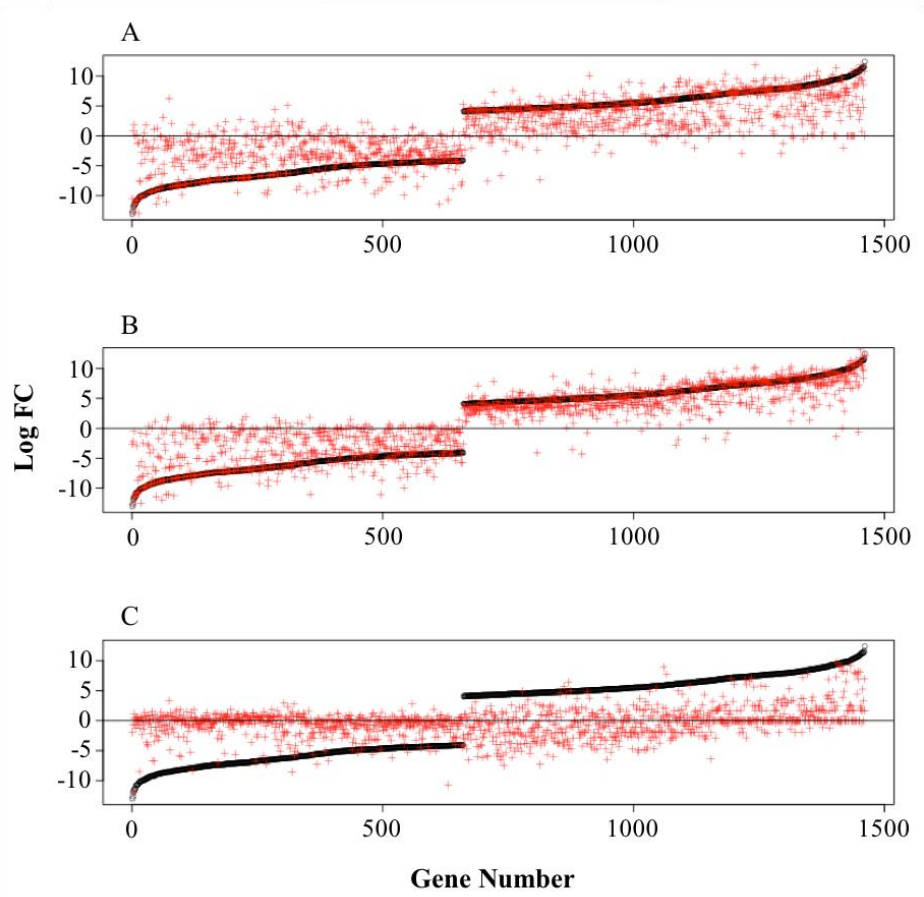

Figure 6: Heatmap of the expression levels of genes of interest in the samples from the single exposure experiment.

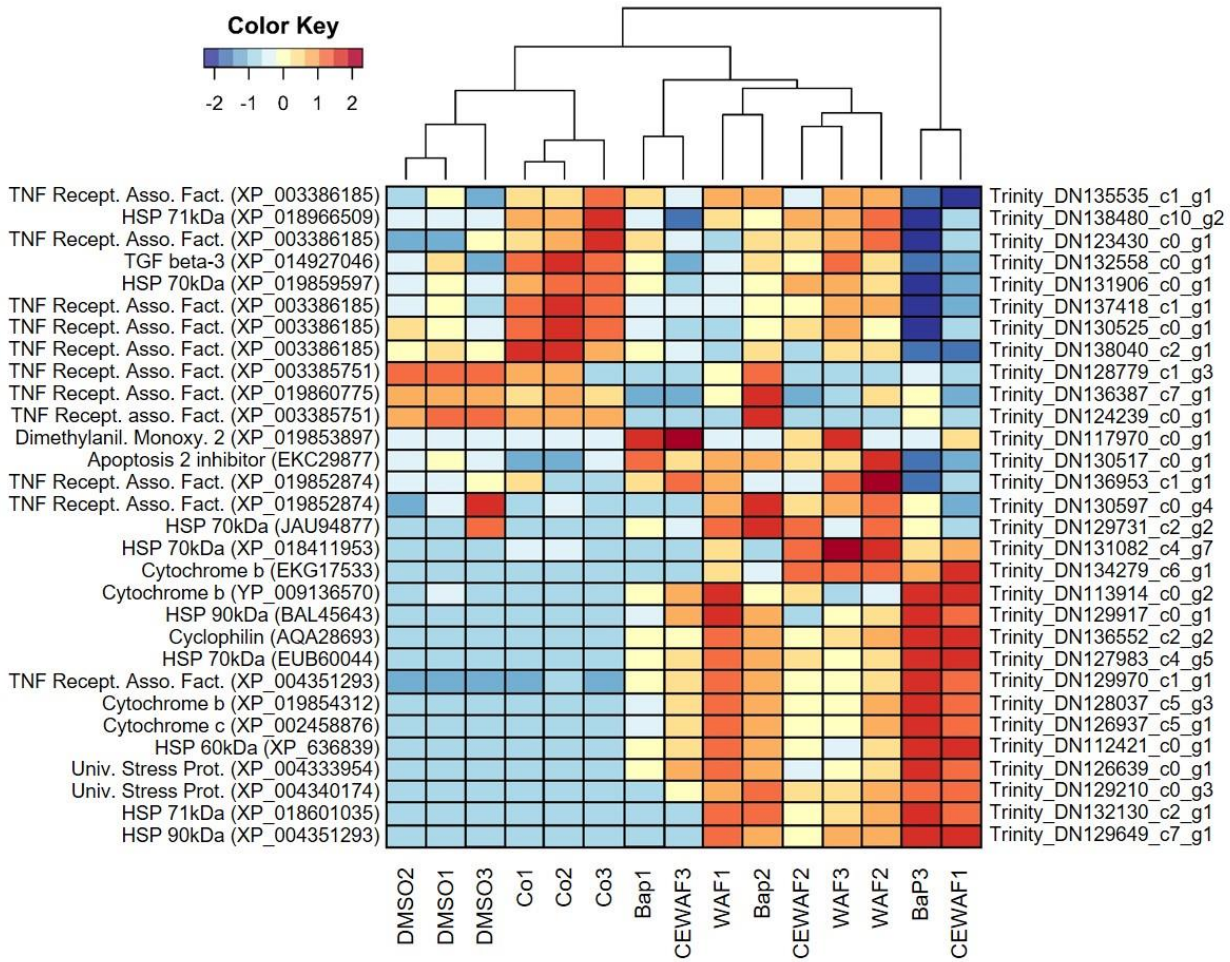

Table 1: ΣPAH_{16} concentrations in µg/L in the single dose experiments and in the dose-response experiment. OL stands for oil loading and is listed in g/L. NA stands for not available.

		ΣPAH_{16}
Single Dose Experiment	WAF	$1.19x10^{-1} \pm 3.74x10^{-2}$
	CEWAF	$2.19x10^{-1} \pm 3.41x10^{-2}$
WAF Dose Response	OL0.01	$8.04x10^{-2} \pm 6.69x10^{-3}$
	OL0.03	$8.81x10^{-2}$
	OL0.05	$4.03x10^{-1}$
	OL0.1	1.37
	OL0.5	$1.54 \pm 1.25x10^{-1}$
	OL1	$1.02x10^{-1}$
	OL5	$1.98 \pm 1.91x10^{-1}$
	OL7.5	26.76 ± 6.78
	OL10	NA
CEWAF Dose Response	OL0.01	$8.53 x10^{-2}$
	OL0.03	NA
	OL0.05	NA
	OL0.1	NA
	OL0.5	$7.71x10^{1} \pm 1.44x10^{1}$
	OL1	NA
	OL5	$5.57 x10^{2}$
	OL7.5	$1.98 \pm 1.91x10^{-1}$
	OL10	$1.38 x10^{4}$

Table 2: Gene families amongst differentially expressed genes in hydrocarbon treatments relative to control relevant for hydrocarbon detoxification and general stress response.

Gene Family	Number of contigs identified
Cyclophilin	1
Cytochrome b	3
Cytochrome c	1
Flavin-containing monooxygenase	1
Heat shock proteins	9
Inhibitor of Apoptosis Proteins	1
MAP3K7 binding protein 1	1
TNF receptor-associated factors	11
Universal stress proteins	2

Supplementary Information

Figure S1: Experimental set-up. (A) Incubation chamber with (1) locking system (2) inflow and (3) magnetic stirrer. (B) Holding Plate with central rotor column controlling the mixing in four adjacent chambers. (C) Experimental set-up with (1) Duran bottles with treatment solutions, (2) Marine Colours peristaltic pump, (3) incubation chambers on holding plate and (4) collection bucket.

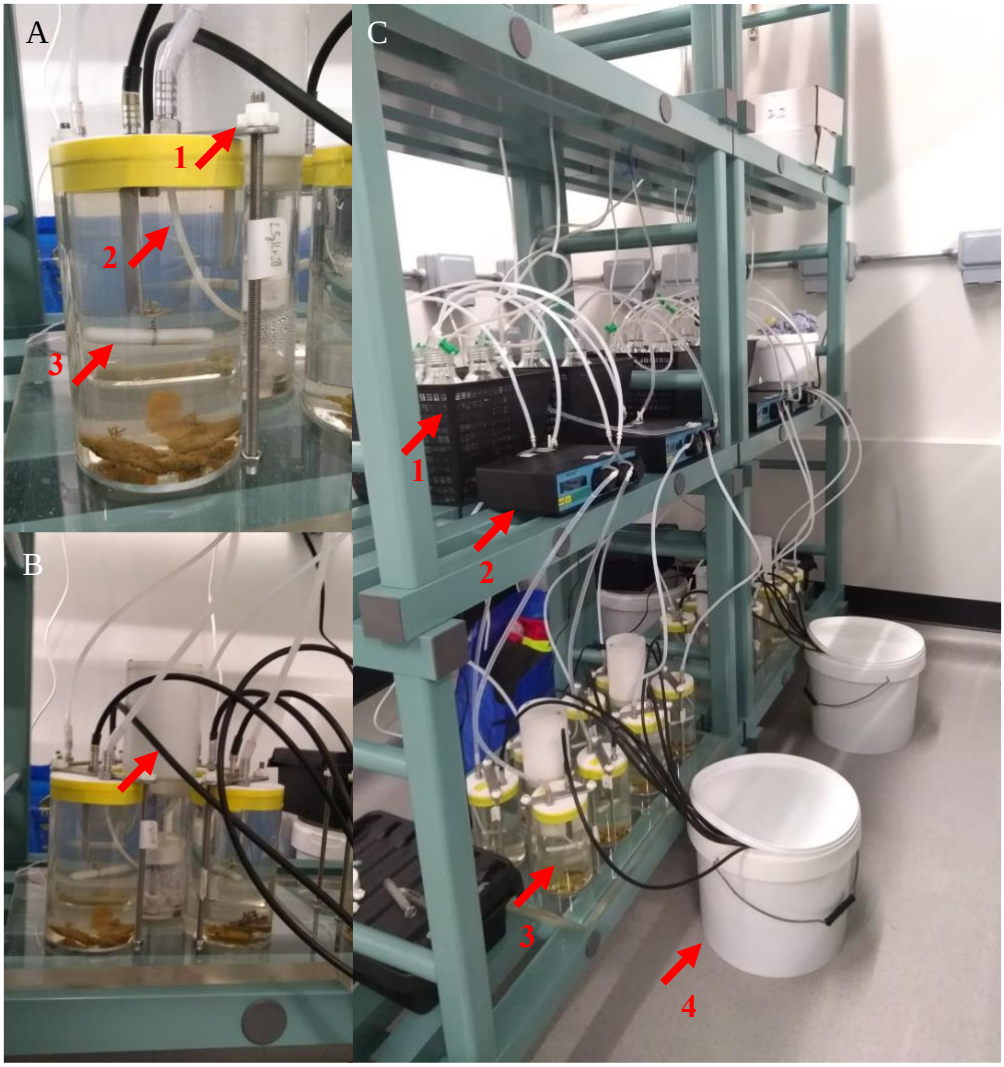

Figure S2: Results of the GO enrichment analysis performed on the up-regulated sponge host genes (GO term, p-value and number of counts is given). Squares in red highlight significantly enriched GO terms.

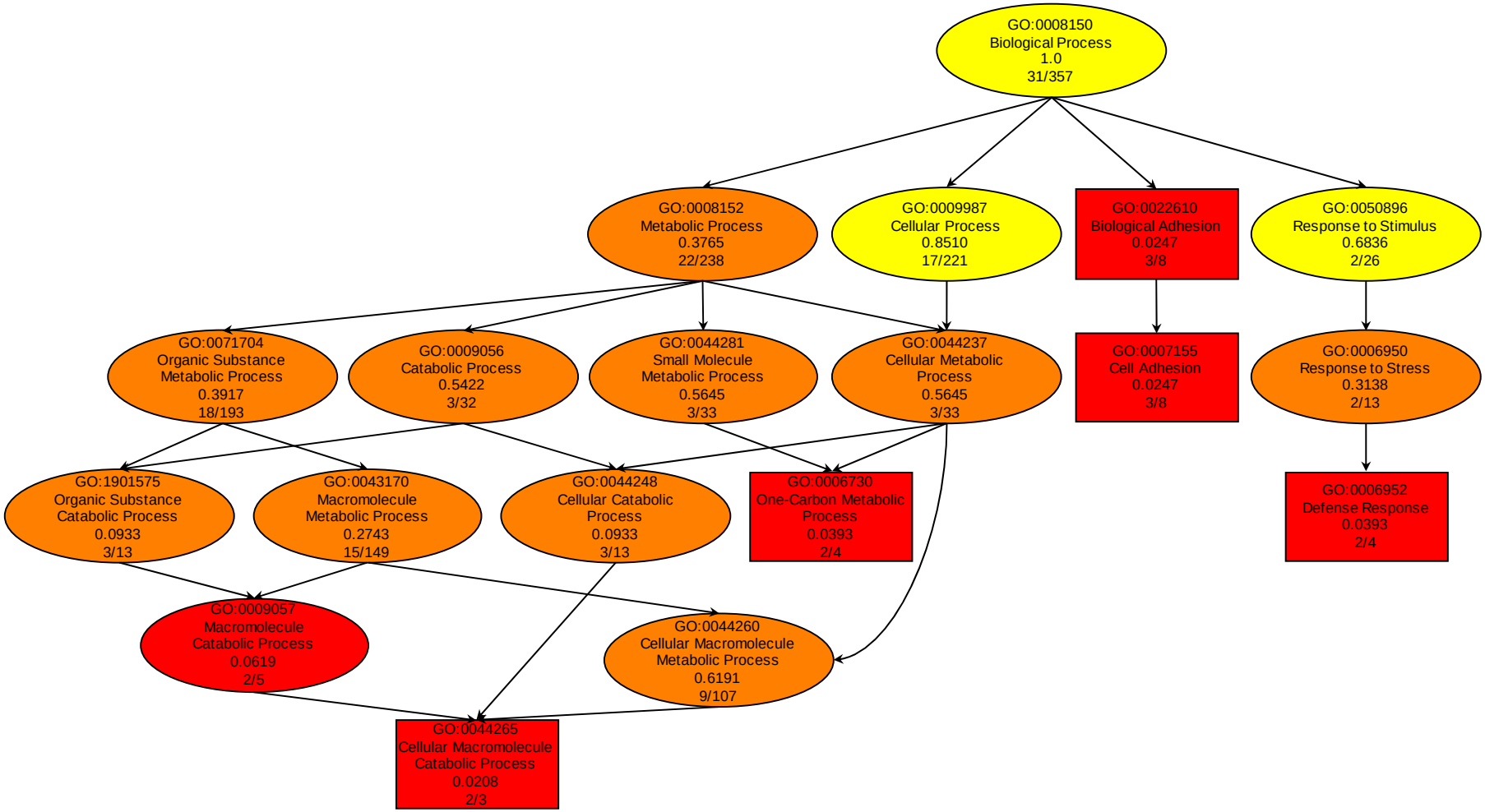

Figure S3: Results of the GO enrichment analysis performed on the down-regulated sponge host genes (GO term, p-value and number of counts is given). Squares in red highlight significantly enriched GO terms.

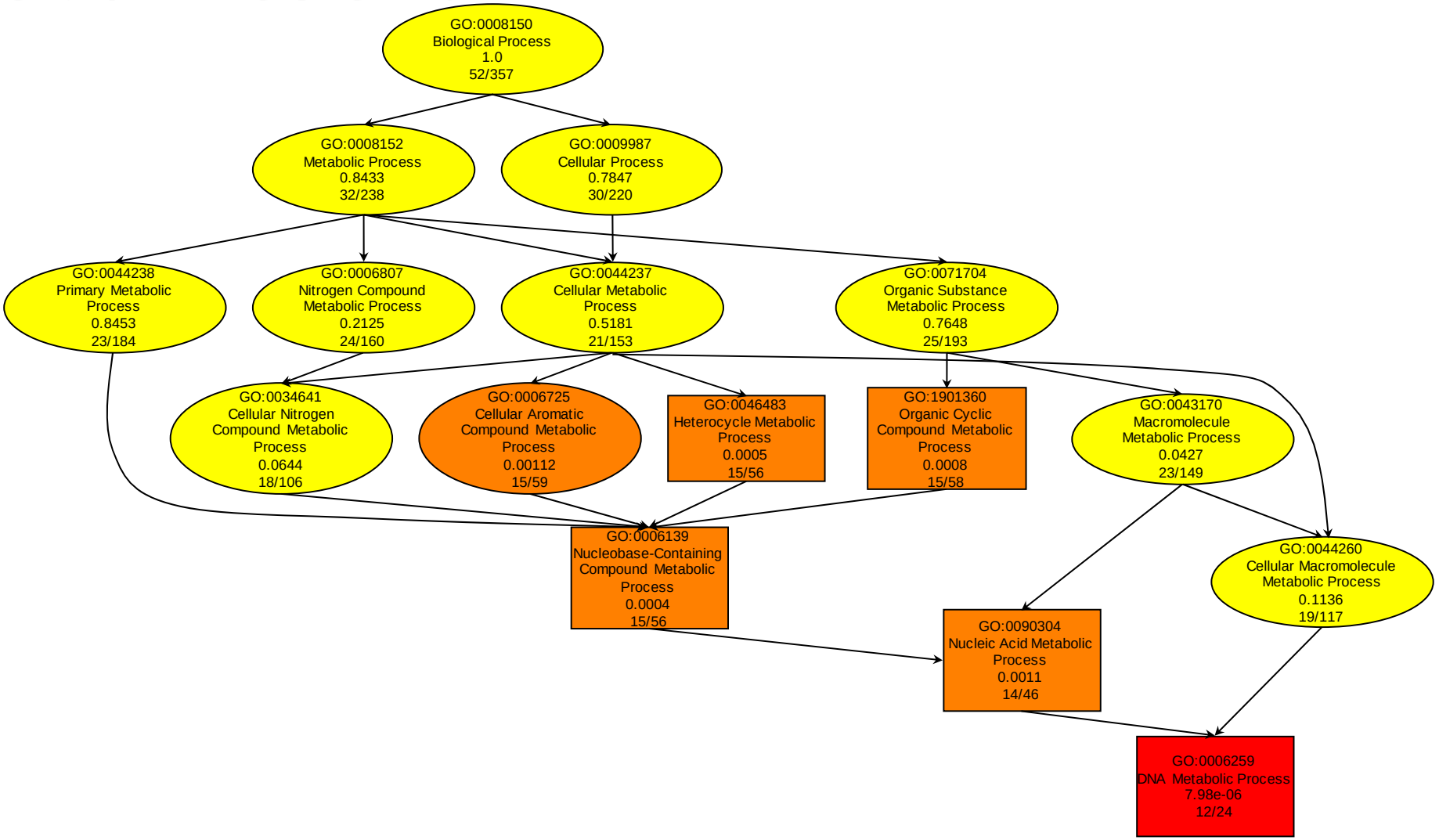

Table S1: Primer sets characteristics. Cyclo means Cyclophilin, Cytb5 Cytochrome b5 and Eff. Efficiency.

Primer	Primer sequence	Position	Tm	Eff.
Cyclo-F	5’-GCGAAGTCGTCGAAGGAATG-3’	445-464	58.5°C	0.973
Cyclo-R	5’-CCTCGATGATGCACCGTTGT-3’	534-515		
Cytb5-F	5’-CCAAGAGATTGCTGGTGGGT-3’	319-338	58.5°C	1.056
Cytb5-R	5’-GGTCATCTGGAGCTCGCATT-3’	531-512		
Hsp70-F	5’-AATTTCTCGTGAGCGGCCTT-3’	274-293	58.5°C	0.9623
Hsp70-R	5’-TCACCTCAGCGAGCAACAAA-3’	381-362		
TK-F	5’-CTCCCAGCTTGCCAAAGAGA-3’	349-368	58.5°C	1.093
TK-R	5’-TGCTAGCACTGGGATTGTGG-3’	635-616		

Table S2: Results of the hydrocarbon analysis for the single concentration experiment. Concentrations are given in μg/L. < LOQ means below limit of detection. NA means not applicable.

	WAF	CEWAF	Average Concentration ratio CEWAF/WAF
Naphthalene	$2.23x10^{-4} \pm 3.87x10^{-4}$	$7.10x10^{-2} \pm 1.62x10^{-2}$	318.39
Acenaphthylene	< LOQ	< LOQ	NA
Acenaphthene	< LOQ	< LOQ	NA
Fluorene	$1.68x10^{-2} \pm 3.69x10^{-3}$	$7.73x10^{-3} \pm 7.34x10^{-3}$	2.17
Phenanthrene	$5.39x10^{-2} \pm 2.33x10^{-2}$	$4.55x10^{-2} \pm 3.52x10^{-3}$	0.84
Anthracene	$8.64x10^{-3} \pm 7.53x10^{-3}$	$1.29x10^{-2} \pm 1.78x10^{-4}$	1.49
Fluoranthene	$2.60x10^{-2} \pm 4.75x10^{-3}$	$3.07x10^{-2} \pm 4.69x10^{-3}$	1.18
Pyrene	$1.38x10^{-2} \pm 7.72x10^{-3}$	$3.83x10^{-2} \pm 1.26x10^{-2}$	2.77
Benzo [A] Anthracene	< LOQ	< LOQ	NA
Chrysene	< LOQ	< LOQ	NA
Benzo [B] Fluoranthene	< LOQ	$1.28x10^{-2} \pm 2.23x10^{-2}$	NA
Benzo [K] Fluoranthene	< LOQ	< LOQ	NA
Benzo [A] Pyrene	< LOQ	< LOQ	NA
Indeno [1,2,3,C,D] Pyrene	< LOQ	< LOQ	NA
Dibenz [A,H] Anthracene	< LOQ	< LOQ	NA
Benzo [G,H,I] Perylene	< LOQ	< LOQ	NA
ΣPAH_{16}	$1.19x10^{-1} \pm 3.74x10^{-2}$	$2.19x10^{-1} \pm 3.41x10^{-2}$	1.84

Table S3: Results of the hydrocarbon analysis for the WAF dose-response experiment. Concentrations are given in µg/L. < LOQ means below limit of detection. NA means not available for analysis.

	Oil Loading (g/L)								
	0.01	0.03	0.05	0.1	0.5	1	5	7.5	10
Naphthalene	$7.24x10^{-3} \pm 6.220x10^{-4}$	$5.09x10^{-3}$	< LOQ	$4.43x10^{-1}$	$4.09x10^{-1} \pm 9.13x10^{-2}$	$5.34x10^{-4}$	$7.37x10^{-1} \pm 1.49x10^{-1}$	$1.12x10^{1} \pm 5.30$	NA
Acenaphthylene	$4.14x10^{-3} \pm 5.85x10^{-3}$	< LOQ	< LOQ	< LOQ	$4.81x10^{-2} \pm 4.65x10^{-2}$	$8.12x10^{-4}$	< LOQ	$6.54x10^{-1} \pm 4.32x10^{-4}$	NA
Acenaphthene	$3.03x10^{-4} \pm 4.28x10^{-4}$	< LOQ	< LOQ	$2.84x10^{-2}$	$6.79x10^{-2} \pm 6.85x10^{-2}$	$4.77x10^{-4}$	< LOQ	$6.78x10^{-1} \pm 1.61x10^{-1}$	NA
Fluorene	$1.55x10^{-2} \pm 2.32x10^{-3}$	$2.60x10^{-2}$	$3.90x10^{-2}$	$1.35x10^{-1}$	$4.46x10^{-1} \pm 1.89x10^{-1}$	$4.34x10^{-4}$	$5.65x10^{-1} \pm 7.79x10^{-2}$	$9.61x10^{-1} \pm 4.06x10^{-2}$	NA
Phenanthrene	$1.52x10^{-2} \pm 3.80x10^{-3}$	$2.70x10^{-2}$	$3.18x10^{-1}$	$4.56x10^{-1}$	$4.02x10^{-1} \pm 1.92x10^{-1}$	$6.13x10^{-1}$	$5.74x10^{-1} \pm 1.12x10^{-1}$	$3.01 \pm 1.24x10^{-1}$	NA
Anthracene	$9.83x10^{-3} \pm 1.39x10^{-2}$	$4.36x10^{-3}$	< LOQ	$1.73x10^{-2}$	$1.52x10^{-2} \pm 1.49x10^{-2}$	$1.57x10^{-2}$	< LOQ	$1.51x10^{-2} \pm 1.24x10^{-2}$	NA
Fluoranthene	$1.58x10^{-2} \pm 1.14x10^{-2}$	$9.19x10^{-3}$	$2.83x10^{-2}$	$5.29x10^{-2}$	$7.85x10^{-2} \pm 1.84x10^{-2}$	$3.67x10^{-2}$	$3.56x10^{-2} \pm 3.20x10^{-3}$	$3.68 \pm 8.40x10^{-1}$	NA
Pyrene	$1.25x10^{-2} \pm 8.99x10^{-3}$	$1.64x10^{-2}$	$1.71x10^{-2}$	$6.53x10^{-2}$	$4.05x10^{-2} \pm 3.41x10^{-2}$	$4.76x10^{-2}$	$6.26x10^{-2} \pm 6.81x10^{-3}$	$5.90 \pm 6.36x10^{-2}$	NA
Benzo [A] Anthracene	< LOQ	< LOQ	< LOQ	< LOQ	$6.28x10^{-2} \pm 1.40x10^{-2}$	< LOQ	< LOQ	$2.68x10^{-1} \pm 3.79x10^{-1}$	NA
Chrysene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	$3.89x10^{-1} \pm 5.50x10^{-1}$	NA
Benzo [B] Fluoranthene	< LOQ	< LOQ	< LOQ	< LOQ	$1.30x10^{-2} \pm 2.92x10^{-2}$	< LOQ	< LOQ	< LOQ	NA
Benzo [K] Fluoranthene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA
Benzo [A] Pyrene	< LOQ	< LOQ	< LOQ	$1.68x10^{-1}$	$1.23x10^{-2} \pm 2.53x10^{-2}$	< LOQ	< LOQ	< LOQ	NA
Indeno [1,2,3,C,D] Pyrene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	$6.72x10^{-3} \pm 9.50x10^{-3}$	NA
Dibenz [A,H] Anthracene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA
Benzo [G,H,I] Perylene	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	NA
ΣPAH_{16}	$8.04x10^{-2} \pm 6.69x10^{-3}$	$8.81x10^{-2}$	$4.03x10^{-1}$	1.37	$1.54 \pm 1.25x10^{-1}$	$1.02x10^{-1}$	$1.98 \pm 1.91x10^{-1}$	26.76 ± 6.78	NA

Table S4: Results of the hydrocarbon analysis for the CEWAF dose-response experiment. Concentrations are given in µg/L. < LOQ means below limit of detection. NA means not available for analysis.

	Oil Loading (g/L)								
	0.01	0.03	0.05	0.1	0.5	1	5	7.5	10
Naphthalene	$6.56x10^{-2}$	NA	NA	NA	$2.01x10^{1} \pm 8.33$	NA	$2.51x10^{2}$	$6.39x10^{2} \pm 2.87x10^{1}$	$1.70x10^{3}$
Acenaphthylene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	$3.35x10^{2} \pm 4.74x10^{2}$	$1.18x10^{3}$
Acenaphthene	$7.88x10^{-3}$	NA	NA	NA	$1.06x10^{1} \pm 4.83$	NA	$1.95x10^{2}$	$1.28x10^{2} \pm 5.69x10^{1}$	$1.02x10^{3}$
Fluorene	$1.35x10^{-3}$	NA	NA	NA	8.16 ± 2.32	NA	$5.38x10^{1}$	$1.69x10^{2} \pm 4.78x10^{1}$	$1.43x10^{3}$
Phenanthrene	$2.22x10^{-2}$	NA	NA	NA	$2.43x10^{1} \pm 1.78$	NA	$2.02x10^{2}$	$8.16x10^{2} \pm 1.16x10^{1}$	$1.45x10^{3}$
Anthracene	$1.80x10^{-2}$	NA	NA	NA	3.16 ± 4.47	NA	6.39	$2.11x10^{2} \pm 7.92x10^{1}$	$2.53x10^{2}$
Fluoranthene	$1.83x10^{-2}$	NA	NA	NA	$1.05x10^{1} \pm 7.36x10^{-1}$	NA	$1.05x10^{1}$	$4.42x10^{1} \pm 5.84$	$9.87x10^{2}$
Pyrene	$1.15x10^{-2}$	NA	NA	NA	$7.73x10-2 \pm 1.09x10^{-1}$	NA	$1.33x10^{1}$	$6.29x10^{1} \pm 8.70$	$9.56x10^{2}$
Benzo [A] Anthracene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	$1.49x10^{2} \pm 6.38x10^{1}$	$1.20x10^{3}$
Chrysene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	$1.31x10^{2} \pm 3.73x10^{1}$	$1.46x10^{3}$
Benzo [B] Fluoranthene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	$7.37x10^{1} \pm 1.27$	$9.90x10^{2}$
Benzo [K] Fluoranthene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	$4.88x10^{1} \pm 6.90x10^{1}$	< LOQ
Benzo [A] Pyrene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	< LOQ	< LOQ
Indeno [1,2,3,C,D] Pyrene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	< LOQ	$1.12x10^{3}$
Dibenz [A,H] Anthracene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	< LOQ	< LOQ
Benzo [G,H,I] Perylene	< LOQ	NA	NA	NA	< LOQ	NA	< LOQ	< LOQ	< LOQ
ΣPAH_{16}	$8.53 x10^{-2}$	NA	NA	NA	$7.71x10^{1} \pm 1.44x10^{1}$	NA	$5.57 x10^{2}$	$1.98 \pm 1.91x10^{-1}$	$1.38 x10^{4}$

Table S5: Results of the repeated-measures ANOVA clearance rate data from the single concentration exposure experiment. Element in bold highlight statistical differences.

Response Variable	Explanatory variable	χ^2	Degrees of Freedom	Pr(>χ^2)
Clearance rate	Treatment	0.8513	4	0.9314
	Time	**31.9094**	**1**	**1.615e-08**
	Treatment*Time	**26.1080**	**4**	**3.010e-05**

Table S6: Results of the dose-response model applied to the clearance rate from the WAF and CEWAF dose-response exposure experiment. Elements in bold highlight statistical differences.

Parameters estimates

	Estimate	Std Error	t-value	P-value
Slope WAF	**0.369172**	**0.120051**	**3.0751**	**0.000104**
ED50 WAF	**1.560996**	**0.482497**	**3.2458**	**0.003609**
Slope CEWAF	**0.326211**	**0.076470**	**4.2659**	**<2.2e-16**
ED50 CEWAF	**0.043311**	**0.018123**	**2.3910**	**0.021145**
Upper Asymptote	**2.229147**	**0.070923**	**31.4304**	**0.002242**

Lack Of Fit Test

Model Degrees of Freedom	RSS	Degrees of Freedom	F value	P-value
44	31.901	17	0.6226	0.8446

No Effect Test

χ^2 test	Degrees of Freedom	P-value
3.431826e+01	**4**	**6.411789e-07**

Table S7: Results of parameter comparison tests between WAF and CEWAF dose-responses

Parameter compared	Estimate	Std Error	t-value	P-value
ED50	**0.027668**	**0.045363**	**-21.434**	**<2.2e-16**
Slope	0.88363	0.87114	-0.3458	0.7311

Table S8: Transcriptomics data summary giving the number of reads before trimming (N reads BT), the number of reads after trimming (N reads AT), the GC content and the sequence length after trimming (Read length AT)

Condition	N reads BT	N reads AT	GC content (%)	Read length AT
Control	51 211 739	41 009 710	47	75
DMSO	58 776 288	47 128 808	48	75
WAF	61 647 651	49 201 885	48	75
CEWAF	56 181 414	44 729 898	48	75
BaP	68 140 135	54 859 604	48	75

Table S9: Statistics of the Trinity *de novo* assembly

Filtered Assembly Statistics	
Total number of contigs	235 561
GC(%)	45.54
N50	984
N75	512
L50	48 509
L75	115 109